MOLECULAR MECHANISMS OF LITHIUM ACTION: CHRONIC LITHIUM REGULATES THE EXPRESSION OF ADENYLATE CYCLASE AND G_i-PROTEIN ALPHA SUBUNIT IN RAT CEREBRAL CORTEX

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1992

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MOLECULAR MECHANISMS OF LITHIUM ACTION: CHRONIC LITHIUM REGULATES THE EXPRESSION OF ADENYLATE CYCLASE AND G_i-PROTEIN ALPHA SUBUNIT IN RAT CEREBRAL CORTEX

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by
Samuel F. Colin
1992

ACKNOWLEDGEMENTS

There are several people who have played an instrumental role in my medical training and thesis preparation. First, I am greatly indebted to Dr. Eric Nestler who has provided superb mentorship during the nearly two years that we have worked together. Without question, the time that I spent in the Laboratory of Molecular Psychiatry has been the most exciting and intellectually stimulating phase of medical school. Eric provided guidance, support, and a level of enthusiasm about this project which helped me to see it through many months of slow progress. His personal excitement was contagious and clearly motivated me and the other medical, graduate, and post-doctoral students in the lab. In addition, he was exceedingly understanding and tolerant of my overnight hot plate experiments. Others have contributed to my scientific research, including the office of student research and the American Heart Association, who generously provided the fellowship which permitted me to pursue laboratory research on a full-time basis.

I feel very fortunate to have had the support of my family and friends throughout my medical school years. My parents have been very helpful in many ways. And, the bonds of friendship that were formed with my medical school cohorts -- Meister, Tilly, Willy, and Charlie -- helped to make this passage a lot more fun than it was supposed to be.

ABSTRACT

A possible role for adenylate cyclase and guanine nucleotide-binding proteins (G-proteins) in contributing to the chronic actions of lithium on brain function was investigated in rat cerebral cortex. Northern blotting and Western blotting techniques were utilized to measure lithium induced changes in mRNA and protein levels of specific adenylate cyclase and G-protein subtypes. It was found that chronic treatment of rats with lithium (with therapeutically relevant serum levels of approximately 1 mM) increased levels of mRNA and protein for the calmodulin-sensitive (type 1) and calmodulin-insensitive (type 2) forms of adenylate cyclase and decreased levels of mRNA and protein for the inhibitory G-protein subunits $G_{i\alpha 1}$ and $G_{i\alpha 2}$. Chronic lithium did not alter levels of other G-protein subunits, including $G_{o\alpha}$, $G_{s\alpha}$, and G_{β}. Lithium regulation of adenylate cyclase and $G_{i\alpha}$ was not seen in response to short-term lithium treatment (with final serum levels of approximately 1 mM) or in response to chronic treatment at a lower dose of lithium (with serum levels of approximately 0.5 mM). The results suggest that up-regulation of adenylate cyclase and down-regulation of $G_{i\alpha}$ could represent part of the molecular mechanism by which lithium alters brain function and exerts its clinical actions in the treatment of affective disorders.

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Portions of this work have been published in the *Proceedings of the National Academy of Sciences USA* (Colin et. al. (1991) *Proc. Natl. Acad. Sci. USA*, 88:10634-10637). Additionally, some of this data has been presented at the 21st Annual Meeting of the Society for Neuroscience, and published in the abstracts of this meeting.

INTRODUCTION

Lithium is the drug of choice for manic-depressive illness. In contrast to most psychotherapeutic agents which acutely affect neurotransmitter receptor activation, lithium appears to act initially upon post-receptor components of signal transduction. However, these acute effects do not correspond with the 2-4 week time course required for lithium's clinical efficacy. We have therefore sought to study the neuronal adaptations which occur in response to chronic lithium therapy in an effort to uncover the critical molecular changes which underlie lithium's therapeutic effect. Before considering the results of our chronic lithium studies, it will be useful to review the cyclic AMP (cAMP) and phophatidylinositol-bis-phosphate (PIP_2) second messenger pathways and the acute actions of lithium on these signal transduction systems.

Transmembrane signalling -- generation of cAMP as a second messenger

Second messenger systems serve the important function of transforming an extracellular signal of a neurotransmitter (or hormone) into an intracellular signal, prompting an intracellular response. The cAMP system is "turned on" when a stimulatory agonist binds its receptor thereby inducing a configurational change. The agonist-receptor complex then interacts with the stimulatory G-protein, G_s, which is a trimer composed of $G_{s\alpha}$, G_β, and G_γ subunits. Upon interaction with the agonist receptor complex, $G_{s\alpha}$ increases its affinity for GTP, and dissociates from the trimer. In the activated GTP-bound state $G_{s\alpha}$ interacts with adenylate cyclase to stimulate the conversion of ATP to cAMP (Fig 1A). The water soluble second messenger, cAMP, binds to the regulatory subunits of cAMP-dependent protein kinase (PKA), composed of 2 regulatory and 2 catalytic subunits. Upon binding 4 molecules of cAMP (2 per regulatory subunit), the 2 catalytic subunits are released thus generating active catalytic subunits (Fig. 1B). The free catalytic subunits

phosphorylate substrate proteins, including ion channels, nuclear proteins, and other protein kinases, which regulate a wide range of cell functions (1).

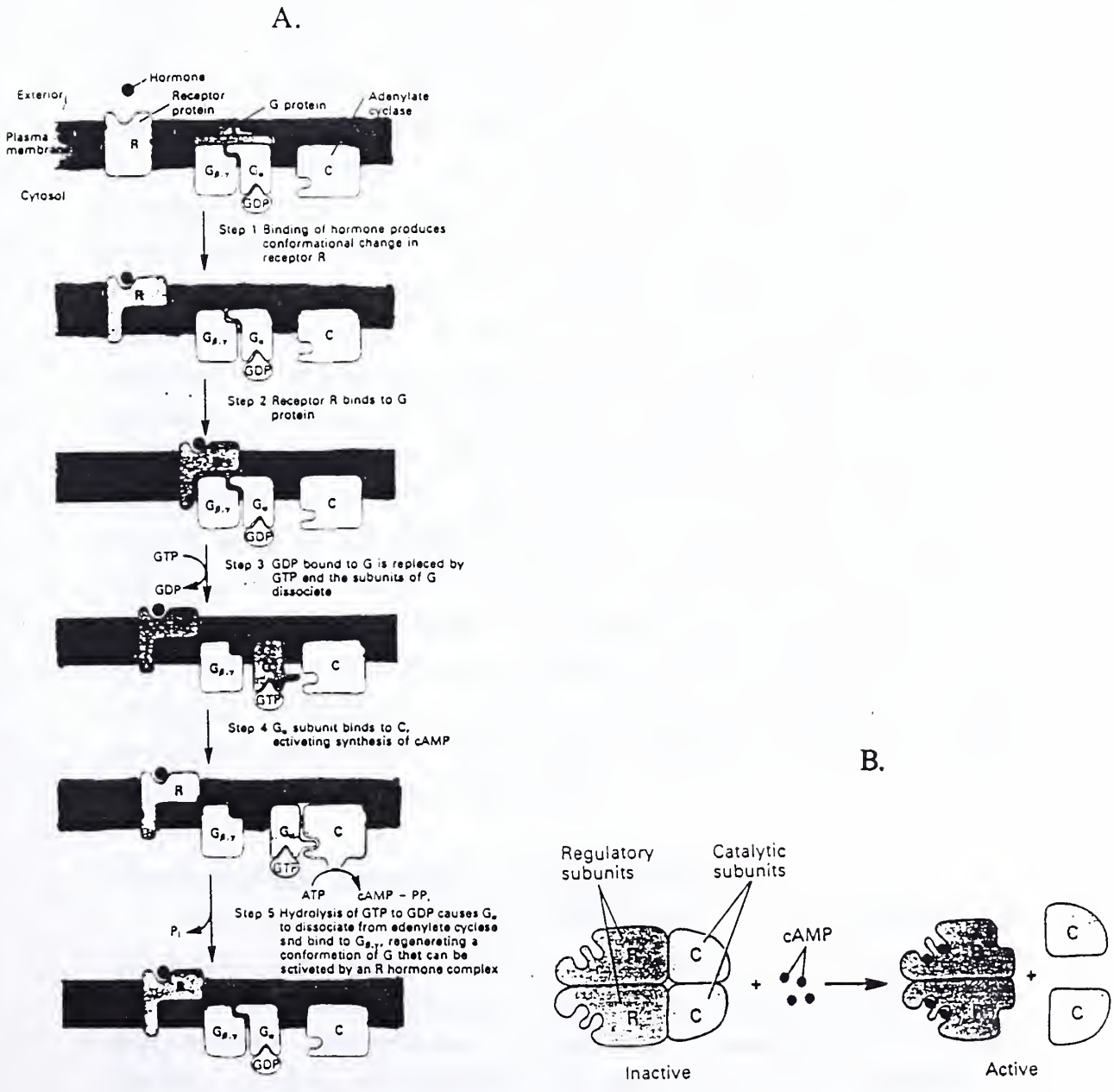

Figure 1. The cAMP signal transduction pathway. A. The stimulatory hormone (neurotransmitter) binds its receptor, activates $G_{s\alpha}$ which stimulates adenylate cyclase to produce cAMP. B. The second messenger, cAMP, binds the regulatory subunits of PKA to liberate 2 active catalytic subunits. Figure adapted from Darnell, J., et. al. (2).

There are greater levels of complexity of cell regulation which extend beyond the scope of this discussion. However, it is important to note that cAMP levels are regulated by the activity of two opposing enzymatic processes -- the conversion of ATP to cAMP by adenylate cyclase versus the degradation of cAMP to AMP by phosphodiesterase (1). There are also counter-regulatory mechanisms at the level of protein phosphorylation -- the phosphorylation of substrate proteins by protein kinases versus dephosphorylation by protein phosphatases (3). In sum, at each level there are homeostatic mechanisms to reverse transient changes in cell stimulation.

Inhibitory agonists act through similar mechanisms as stimulatory agonists. However the exact mechanism of G_i inhibition of adenylate cyclase has not been clearly established. Inhibitory agonists bind inhibitory receptors and the agonist-receptor complex interacts with G_i inducing $G_{i\alpha}$ binding of GTP and dissociation of the $\alpha-\beta-\gamma$ trimer. It is unclear whether $G_{i\alpha}$ directly inhibits adenylate cyclase or whether the liberation of free $G_{\beta\gamma}$ dimers sequesters active $G_{s\alpha}$; this would indirectly inhibit adenylate cyclase by suppressing its activation from $G_{s\alpha}$. Through either direct or indirect means, stimulation of G_i decreases adenylate cyclase activity, decreases cAMP production, and thereby allows the regulatory subunits of PKA to inhibit the catalytic subunit (1).

Transmembrane signalling -- PIP_2 hydrolysis

The receptor mediated stimulation of phosphoinositide hydrolysis has recently been elucidated as a second major pathway of transmembrane signalling. Phosphoinositides are phospholipid components of the cell membrane. PIP_2 is a member of this phospholipid family and is the substrate for generation of second messengers in this system. Receptor stimulation appears to activate phospholipase C (PLC) via the recently identified G_q class of G-proteins (3a). PLC hydrolyzes PIP_2 into two products, water soluble inositol triphosphate (IP_3) and diacylglycerol (DG) (4). IP_3 acts upon the intracellular

IP_3 receptor to liberate internal calcium stores. The elevation of cytoplasmic calcium concentration activates a variety of processes through the calcium binding protein, calmodulin, including stimulation of calcium-calmodulin dependent protein kinases (4). The lipophilic DG remains associated with the membrane where it interacts with protein kinase C (PKC). Activation of PKC is dependent on both the availability of DG and free calcium (Fig. 2). Similar to PKA, this enzyme phosphorylates a variety of substrate proteins and thereby regulates cell function (4).

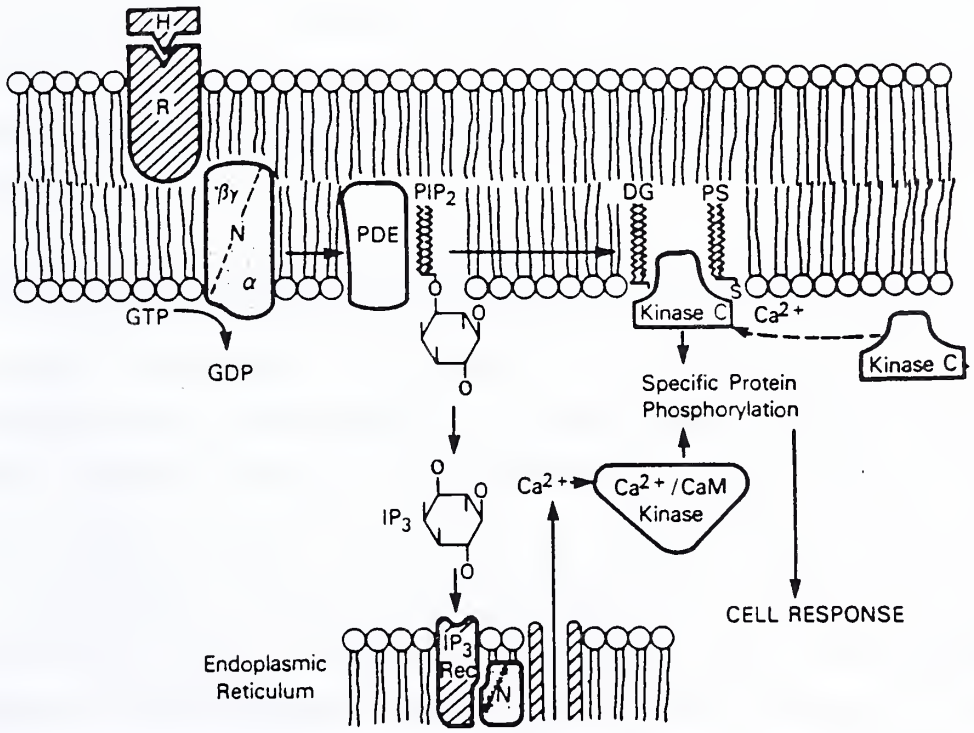

Figure 2. The PIP_2 hydrolysis signal transduction pathway. The stimulatory agonist binds its receptor and activates PLC which hydrolyzes PIP_2 to form the second messengers DG and IP_3. Both PKC and calcium-calmodulin dependent protein kinases are activated in this cascade of events. Figure adapted from Catt, K.J. (5).

The genetics of transmembrane signalling

As both the cAMP and PIP_2 hydrolysis second messenger systems are vital components of cell regulation, the constituent proteins of these systems have been the target of many gene cloning experiments. One of the great surprises from these efforts has been the multiplicity of distinct genes encoding distinct subtypes of many of these signal transducing proteins including, G-proteins, adenylate cyclase, regulatory and catalytic subunits of PKA, PLC, and PKC. One functional consequence of this heterogeneity is that different tissues may respond to extracellular stimuli with different signal transducing machinery, and thereby have heterogeneous responsiveness to identical stimuli. Previous hypotheses had focused on heterogeneity at the receptor level as the major source of differential stimulus-response coupling in different tissues.

From second messengers to regulation of gene transcription

One of the central questions in biology is how cells exercise differential regulation of gene transcription. This question was first addressed in prokaryotes and has recently been studied in great depth in eukaryotic cells. There are two main aspects to this subject -- (i) tissue specific gene transcription (ie. the mechanisms by which a tissue can express a unique set of genes) and (ii) hormonal regulation of gene expression. We will focus on the latter topic with particular regard to cell surface signalling and the regulation of gene transcription by both the cAMP and PIP_2 second messenger systems.

Upon activation of the cAMP cascade, the catalytic subunit of PKA phosphorylates a myriad of cytoplasmic proteins and, in addition, translocates to the nucleus where it phosphorylates the cAMP regulatory element binding protein (CREB) at a single site, Ser-133 (6). Phosphorylation of CREB promotes mRNA transcription from genes which contain the cAMP regulatory element (CRE, 5'-TGACGTCA-3') in the

transcriptional promoter region (6). It is important to note that the presence of a CRE in the promoter region of a gene confers cAMP-inducibility to the gene. It is believed that when CREB binds the CRE it promotes formation of a transcriptional initiation complex at the TATA box -- at approximately -30 in a majority of eukaryotic genes (7). This process takes place in a stepwise fashion with TFIID being the first subunit of RNA polymerase II to bind in the TATA region, followed by IIA, IIB, and IIE, to form a competent transcription complex. In fact, the enhancement of the TFIID-DNA interaction is believed to be a general mechanism whereby transcriptionally active proteins (via an α-helical acidic activating region) promote gene transcription (6). In sum, stimulation of the cAMP cascade directly regulates specific gene transcription of CRE-containing genes through PKA phosphorylation of CREB.

The stimulation of PIP_2 hydrolysis results in IP_3-mediated rises in cytosolic Ca^{++} and PKC phosphorylation of substrate proteins. Activation of this second messenger pathway is believed to induce gene transcription by at least two mechanisms: (i) elevation of internal Ca^{++} leads to calmodulin-dependent phosphorylation of CREB (presumably via calmodulin-dependent protein kinase) and (ii) PKC activates transcription of the nuclear protein c-fos. In the case of PKC induction of c-fos it appears that PKC activates serum response factor (SRF), a transcriptionally active 67 kDa nuclear protein (6). Upon activation, SRF binds the serum response element (SRE) which is located at -310 in the c-fos gene, and thereby increases the rate of c-fos transcription (6). However, the mechanism through which PKC activates SRF is unknown. There is cross-talk between PKA-mediated and PKC-mediated events at the level of c-fos induction -- the c-fos gene is induced by PKA, via CREB phosphorylation, which enhances transcription by binding the CRE located at -60 in the c-fos gene (6). In addition, Ca^{++} can also induce transcriptional activation of c-fos at the same CRE site, presumably by calmodulin-dependent protein kinase phosphorylation of CREB (6). In sum, cAMP, DAG,

and Ca^{++} can independently activate c-fos transcription. In this manner the second messengers couple cellular stimulation to the regulation of c-fos transcription. We will now focus on c-fos as a regulator of gene transcription.

Upon cell stimulation, c-fos mRNA levels rise within 30-45 minutes via the previously described second messenger systems. The c-fos protein is a 62 kDa nuclear protein that undergoes extreme post-translational modification (6). There are in fact several fos-related genes which have been cloned and sequenced. They all share regions of homology including the DNA binding domain and leucine-zipper region. This region is characterized by an interaction of leucine side chains, spaced 7 amino acids apart, on adjacent α-helices (6). Through the leucine zipper fos binds another nuclear protein, jun, to form the transcriptionally active AP-1 complex. Genes which contain the AP-1 recognition site (5'-TGACTCA-3'), also known as the TPA recognition element (TRE), can be induced by AP-1 (6). In a similar manner to the CREB dimer, AP-1 utilizes an acidic activating region to promote RNA polymerase II activity at the TATA box, and thereby stimulates gene transcription. In sum, cAMP directly activates transcription from CRE-containing genes and indirectly from TRE-containing genes via fos. In contrast, PIP_2 hydrolysis acts indirectly via SRF-mediated fos induction to stimulate transcription from genes possessing TRE sites. As lithium acutely inhibits both the cAMP and PIP_2 hydrolysis pathways it will be important to consider the link between second messengers and gene transcription as a mechanism by which chronic lithium regulates gene expression.

Acute lithium effects on the cAMP system

The most consistently reproduced finding in lithium research is that lithium acutely inhibits neurotransmitter (hormone)-, forskolin-, and guanine nucleotide-stimulated adenylate cyclase activity in both peripheral tissues and brain

(8-10). Interestingly, the persistence of this short term effect is clinically manifested in the most common side effect of lithium therapy, nephrogenic diabetes insipidus. In the collecting duct of the kidney, antidiuretic hormone (ADH) binds to receptors and stimulates an increased permeability to water and thereby decreases urine volume. Transmembrane signalling of ADH occurs via the stimulation of adenylate cyclase, increasing the production of cAMP. Lithium inhibits ADH stimulated cAMP production and thereby blocks ADH induced water resorption, with the net effect of creating a diuresis (11). Another side effect of lithium therapy, hypothyroidism, is caused by a similar mechanism. Lithium blocks thyroid stimulating hormone (TSH) induced cAMP production in the thyroid and thereby attenuates TSH-stimulated thyroxine synthesis and release (12). In addition to these clinically important side effects, lithium inhibits adenylate cyclase in a variety of other peripheral tissues including prostaglandin E_1-sensitive adenylate cyclase in human platelets, beta-adrenergic-sensitive cyclase in human lymphocytes, and epinephrine-sensitive cyclase in skeletal muscle (9, 13).

Some of the earliest evidence for lithium's acute inhibition of adenylate cyclase activity in rat brain was derived by *in vitro* studies. Forn and Valdecasas demonstrated that the addition of exogenous lithium (2 mM) to both cortical slices and homogenates significantly decreased the ability of norepinephrine (NE) to stimulate adenylate cyclase activity (14). The same effect was observed when NaF was used to stimulate the enzyme. These studies pre-date the discovery of G-proteins and the insight that NaF directly activates $G_{s\alpha}$; however, in retrospect, the authors clearly demonstrated that lithium acutely blocks adenylate cyclase activation at the post-receptor level. The major criticism of these studies is the lack of data at more clinically relevant lithium concentrations (ie. .75 mM - 1.5 mM). Nevertheless, these early studies provided much of the focus on adenylate cyclase as a target for lithium action.

Later studies corroborated that lithium inhibits adenylate cyclase activation in brain. Ebstein et. al. (1980) demonstrated that lithium inhibits NE-induced cAMP accumulation in synaptosomes and slices from rat cortex (9). In addition, these studies demonstrated that other ionic elements, rhubidium and cesium, do not exert this effect.

More recent studies have approached the question of lithium action with a much better understanding of signal transduction and a better arsenal of pharmacologic agents which act directly on G-proteins. Newman and Belmaker demonstrated that Mn^{++}, forskolin, and GppNHp activation of adenylate cyclase were significantly inhibited by lithium in cortical membranes (8). Interestingly, inhibition of the activity stimulated by forskolin and GppNHp was competitively reversible in the presence of Mg^{++}. The emerging picture from these investigations is that lithium acts at the post-receptor level of G-proteins and/or adenylate cyclase and that this action is reversible in the presence of Mg^{++}.

Lithium's attenuation of receptor activated adenylate cyclase stimulation raises the question of a putative site and mechanism for lithium's acute actions. Mork and Geisler have studied the effects of lithium on the stimulation kinetics of isolated adenylate cyclase from solubilized rat brain (15). These studies confirm that lithium inhibits calcium- and forskolin- stimulated adenylate cyclase activity. Once again, lithium's inhibitory effect was blocked by the presence of excess Mg^{++} (15). These findings raise the possibility that lithium displaces Mg^{++} from its binding site on adenylate cyclase where Mg^{++} is believed to aid in binding ATP for conversion to cAMP.

In more recent attempts to define a post-receptor site of lithium action, Avissar et. al. demonstrated that lithium attenuates agonist stimulated GTP binding by cholera toxin- and pertussis toxin-sensitive G proteins *in vitro* and *in vivo* (16). This effect persists with chronic lithium treatment *in vivo*. These data are quite remarkable in that agonist

stimulated GTP binding is completely abolished in the presence of therapeutic concentrations of lithium. These studies suggest that lithium acts directly on G-proteins to attenuate the actions of receptor agonists. It is possible that lithium acts at a nucleotide triphosphate binding site on G-proteins, similar to the ATP-binding site on adenylate cyclase, to interfere with G-protein function. It is interesting to consider that the cloned sequence of adenylate cyclase (type 1) contains a region near the amino terminus which is identical to the highly conserved region of the guanine nucleotide binding site on G-proteins (17). Therefore lithium may act in a similar manner on both G-protein and adenylate cyclase, by displacing Mg^{++}, thereby preventing protein-nucleotide triphosphate interaction. In sum, lithium appears to act at both the level of G-proteins and adenylate cyclase to inhibit the synthesis of cAMP in response to neurotransmitter stimulation.

Acute lithium effects on the PIP_2 system

A widely held view of lithium action is based on its effects on the PIP_2 hydrolysis second messenger pathway. According to the inositol depletion hypothesis, lithium acutely inhibits inositol phosphate phosphatases, thereby preventing the recycling of free inositol for *de novo* PIP_2 synthesis (Fig. 3) (18). Upon hydrolysis from PIP_2, IP_3 can undergo two different fates -- further phosphorylation to form IP_4, IP_5, and IP_6 (whose functions as intracellular messengers are currently being investigated) or dephosphorylation to different forms of inositol biphosphates and inositol monophosphates. In order to regenerate free inositol for novel PIP_2 synthesis, it is necessary for the inositol monophosphates to undergo dephosphorylation by the enzyme inositol monophosphate phosphatase. Lithium acutely inhibits this reaction. The question which arises is whether this prevents *de novo* PIP_2 synthesis as free inositol is available in the extracellular environment. The proponents of the inositol depletion hypothesis contend that neural tissue is unique because there is minimal transport of free inositol

across the blood-brain barrier. In the proposed absence of both extracellular and intracellular free inositol, neural tissue supposedly becomes depleted of the membrane phospholipid PIP_2 (18).

Perhaps the best evidence supporting this hypothesis is the finding that lithium treatment results in an accumulation of the precursor molecule CMP-PA which combines with free inositol to form PIP_2 (ie. inositol levels supposedly become insufficient to utilize normal amounts of CMP-PA in the synthesis of novel PIP_2) (18). The authors also point to the fact that lithium treated tissues have decreased agonist-stimulated PIP_2 hydrolysis.

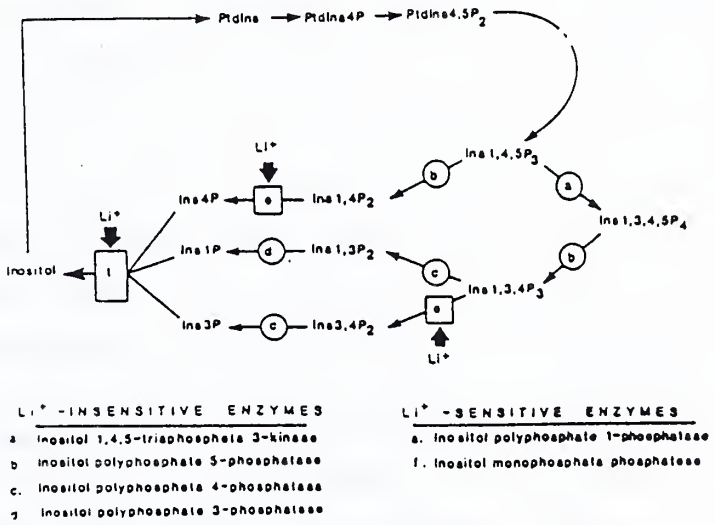

Figure 3. The inositol depletion hypothesis. Lithium blocks key enzymes in the recycling of free inositol from inositol polyphosphates and monophosphates. The proponents of this theory contend that lithium thereby depletes neuronal membranes of PIP_2 needed for transmembrane signalling. Figure adapted from Berridge et. al. (18).

The basic tenet of the inositol depletion hypothesis is that chronic lithium therapy is proposed to deplete neuronal membranes of PIP_2 and thereby interfere with neurotransmission. However, most of the available evidence strongly contradicts this theory. In fact Godfrey et. al. have demonstrated a significant increase in PIP_2 levels in rat

cerebral cortex after lithium therapy (19). Other studies have found that chronic lithium slightly increases or does not affect PIP_2 levels in rat cortex (20). In the absence of any evidence to directly support the hypothesized lithium induced depletion of brain PIP_2 it is important to search for alternative explanations of lithium's actions. It is very possible that other mechanisms may involve lithium regulation of PIP_2 hydrolysis independent of its effects on the inositol monophosphate and polyphosphate phosphatases.

In an analagous manner to lithium's attenuation of cAMP production, lithium appears to dampen agonist stimulated PIP_2 hydrolysis. Kendall and Nahorski found that therapeutic levels of lithium acutely decrease muscarinic stimulated PIP_2 hydrolysis by approximately 60% in rat cortex (21). This effect persists with chronic treatment. Interestingly, when chronically treated rats are withdrawn from lithium for 18 hours the response to carbachol is significantly increased with respect to controls. The molecular basis of this rebound effect is unknown, but the data suggest that chronic lithium may upregulate one or more components in the PIP_2 cycle and that upon lithium withdrawal this hypersensitive system is unmasked. Chronic lithium inhibition of receptor-coupled inositol phospholipid hydrolysis has been corroborated in rat cortex as well as hippocampus, and striatum (22). Similar findings of lithium acutely inhibiting inositol phosphate formation have been reported in mouse cortex (23). In sum, these studies suggest that lithium acutely inhibits phosphoinositide hydrolysis and that this effect persists with chronic administration.

The electrophysiological consequences of acute lithium inhibition of PIP_2 hydrolysis were studied in an elegant set of experiments performed by Worley et. al. . This study found that lithium blocks a phosphoinositide-mediated cholinergic response in hippocampal slices (24). In hippocampus, adenosine inhibits depolarization, but this effect can be blocked by muscarinic stimulation which is mediated by PIP_2 hydrolysis. Muscarinic stimulation is blocked by therapeutic

levels of lithium, presumably secondary to lithium inhibition of muscarinic stimulated PIP_2 hydrolysis. In the presence of lithium, the PKC agonist, TPA, can restore the normal muscarinic response. These experiments suggest that lithium inhibits muscarinic PIP_2 hydrolysis and that this can be bypassed by TPA-stimulation of PKC (24).

In sum, there is substantial evidence to suggest that lithium acutely attenuates neurotransmitter-stimulated PIP_2 hydrolysis and that this blockade of the second messenger system attenuates the electrophysiological response to the neurotransmitter. As previously discussed, lithium acutely inhibits agonist stimulated G-protein-GTP binding (16). One interesting possibility is that lithium blocks PIP_2 hydrolysis by attenuating the function of the G-protein (ie. G_o or G_i) which couples the receptor to PLC activation.

Lithium regulation of gene expression -- focus on signal transducing proteins

In consideration of past work on the acute effects of lithium in signal transduction, we studied the chronic adaptations which occur in response to the lithium induced perturbations in neuronal function. As regulation of gene expression is a crucial mechanism of cellular adaptation, we investigated the effects of chronic lithium on the expression of genes involved in signal transduction, specifically those genes encoding signal transducing proteins that are acutely affected by lithium -- adenylate cyclase and G-proteins. The validity of our rationale is supported by past studies of the long term actions of other types of psychotropic drugs including anti-depressants, anti-psychotics, morphine, and cocaine. These drugs produce chronic adaptations in some of the signal-transduction proteins that are regulated by the drugs acutely (25-28). Although there has been much interest in interactions between lithium and cAMP signal transduction, it is only with the recent availability of cDNA clones and monoclonal

antibodies for adenylate cyclase that our studies could be conducted.

Our approach to the *in vivo* study of gene expression compares changes at both the mRNA and protein level using Northern blotting and Western blotting procedures (see Methods). We believe it is essential to demonstrate that a drug induced change in mRNA is corroborated by a similar change in the encoded protein in order for the effect to have functional significance. Furthermore, chronic lithium induced changes in gene expression were evaluated in terms of both dose dependence and time dependence. Clearly the most clinically important changes are those that are dependent upon a therapeutic dose and time course.

METHODS

Lithium administration

Male Sprague-Dawley rats (initial weight, 140-160 g) were fed pellets containing 0.24% (wt/wt) lithium carbonate (Teklad, Madison, WI) for either 6 days ("short-term lithium") or 4 weeks ("therapeutic lithium") according to established protocols (29). In some experiments, rats were fed 0.17% lithium chloride for 4 weeks ("chronic low-dose lithium"). Serum lithium concentrations were measured by the clinical laboratories of Yale-New Haven Hospital. The average lithium levels from the short-term, therapeutic, and chronic low-dose feeding regimens were 0.98 $\pm$ 0.18, 1.1 $\pm$ 0.25, and 0.57 $\pm$ 0.14 mM, respectively. The 6-day treatment period for the short-term regimen was chosen as the time when serum lithium concentrations have reached steady-state (ie., therapeutic) levels but when the animal has been exposed to such levels for a short time only. The short-term and chronic low-dose lithium treatments were utilized to assess the time and dose dependence of lithium regulation of adenylate cyclase and G-proteins. Control animals were fed the identical diets without added lithium. Normal drinking water and hypertonic saline (1.5%) were available to all rats ad libitum; this has been shown to avoid dehydration in previous investigations (29), and there were no signs of dehydration in the current study. Rats that received chronic lithium gained weight at a slower rate than control rats: from an initial weight of 150 g, control and lithium-treated rats weighed, at the end of the 4-week treatment period, 250 and 330 g, respectively. However, this lithium-induced retarded weight gain *per se* cannot account for the observed regulation of adenylate cyclase and G proteins, as chronic treatment of rats with a number of other psychotropic drugs (which lead to similar degrees of retarded weight gain) do not produce these effects (27, 28, 30 and unpublished observations). Lithium administration was performed by S.C. .

Isolation of RNA and Northern blotting

At the end of the treatment protocols, cerebral cortex was removed rapidly from decapitated animals. Total RNA was isolated by guanidinium isothiocyanate extraction followed by ultracentrifugation in cesium chloride exactly as described (30). Northern blot analysis of adenylate cyclase type 1, adenylate cyclase type 2, and G-protein subunit mRNA was carried out using 20 μg, 10 μg, and 10 μg, respectively, of total RNA per lane in a 1% agarose gel. Other mRNA types studied include PKC subtypes β1, γ, PKA subunit RIβ, and c-fos, all of which required 10 μg of total RNA per lane. The PKA subunit Cα and the nuclear protein sp1 required 20 μg of total RNA per lane. RNA in the gels was transferred to nitrocellulose filters, which were then hybridized to cDNA probes (labelled with ^{32}P by the random primer method; Amersham) for 16 hr at 42°C in the presence of 1% (vol/vol) formamide. The cDNA probes used for adenylate cyclase and G-proteins are described elsewhere (17, 31, and unpublished observations). The probes for adenylate cyclase type 1 and type 2 each recognized single bands of approximately 11.5 and 4.1 kilobases (kb), respectively, as found in previous studies (17). The probes for G-protein subunits also recognized specific bands as reported previously ($G_{i\alpha1}$, 3.5 kb; $G_{i\alpha2}$, 2.4 kb; $G_{i\alpha3}$, 3.5 kb; $G_{s\alpha}$, 1.9 kb; $G_{o\alpha}$ 4.5 and 4.1 kb; and G_{β}, 3.0 kb) (30, 31). The probes for PKC subtypes β1 and γ (kindly provided by John Knopf, Genetics Institute) each recognized a 3.5 kb band as previously reported (32). The probes for PKA subunits Cα and RIβ (kindly provided by Stanley McKnight, University of Washington, Seattle) recognized 2.4 kb and 2.8 kb bands respectively as previously reported (33, 34). The probes for c-fos (kindly provided by Tom Curran, Roche Institute, New Jersey) and sp1 (kindly provided by Robert Tjian, University of California, Berkeley) recognized 2.2 kb and 8.2 kb bands respectively as previously reported (35,36). All Northern blots were subsequently reprobed with a random-prime ^{32}P-labelled cDNA probe for

cyclophilin (kindly provided by J.G. Sutcliffe, Research Institute of Scripps Clinic) (37). Resulting blots were dried and autoradiographed. Levels of adenylate cyclase and G-protein mRNA were quantified by computerized laser densitometry (Pharmacia) of autoradiograms or by Betagen (Waltham, MA) Betascope analysis of the original blots and normalized to cyclophilin mRNA levels by the same instrument. Isolation of RNA, Northern blotting, and autoradiography was performed by S.C. .

Immunoblotting

For adenylate cyclase immunolabelling, cerebral cortex was homogenized (20 mg wet weight per ml) in ice-cold 20 mM Tris, pH 7.4/1 mM dithiothreitol/1 mM EGTA containing 50 kallikrein-inhibitor units of aprotinin (Sigma) and 10 µg of leupeptin (Sigma) per ml. Homogenates were centrifuged at 10,000 x g in a refrigerated microcentrifuge for 10 min and pellets were resuspended in 1% SDS. Protein content was assayed by the method of Lowry et. al. (38). Aliquots of resuspended pellets (containing 150-300 µg of protein) were subjected to SDS/polyacrylamide gel electrophoresis with 5% acrylamide/0.13% N,N' -methylenebisacrylamide in the resolving gels. Proteins were transferred electrophoretically to nitrocellulose filters in the presence of 0.025% SDS, and resulting filters were immunolabelled for adenylate cyclase as described (27). All blotting buffers contained 150 mM NaCl, 20 mM sodium phosphate (pH 7.4), 0.05% Tween (Sigma), and 0.5% (wt/vol) nonfat dry milk. A monoclonal antibody (BBC-2; 1:5000 dilution) prepared against purified adenylate cyclase from bovine cerebral cortex (39) and ^{125}I-labelled goat anti-mouse immunoglobulin (1000 cpm/µl; New England Nuclear) were used in these studies. Resulting blots were dried and autoradiographed. These conditions recognized two major bands of about 150 and 130 kDa, plus a smear between the bands. The following lines of evidence support the view that this represented specific labelling of adenylate cyclase: (i) the

labelling was enriched in membrane fractions compared with crude homogenates and was fully solubilized by 10 mM Lubrol (Sigma) as described previously for adenylate cyclase (39); (ii) the labelling was greatly enriched in fractions of neostriatum compared with cortex, as is the case for adenylate cyclase (39, 40); and (iii) the pattern of labelling resembled that reported previously for cerebral cortex (39).

For G-protein immunolabelling, crude homogenates of cerebral cortex were adjusted to contain 1% SDS and their protein content was determined. Aliquots (10-50 μg of protein) were subjected to SDS/polyacrylamide gel electrophoresis (with 9% acrylamide/0.24% N,N' -methylenebisacrylamide in the resolving gels) and to immunolabelling for G-protein subunits exactly as described (27,30). Rabbit polyclonal antisera, either purchased from New England Nuclear ($G_{i\alpha 1/2}$) or kindly provided by John Northrup of Yale University (G_{β}), and ^{125}I-labelled goat anti-rabbit IgG (300 cpm/μl; New England Nuclear) were used in these studies. Levels of G-protein immunoreactivity were quantified by densitometry. These conditions specifically label G-protein subunits ($G_{i\alpha 1/2}$, 40-41kDa; G_{β}, 35-36 kDa) and result in linear levels of immunoreactivity over a 5-fold range of tissue concentration (27,30). Preparation of homogenates and immunoblotting was performed by Rose Terwilliger, E.J.N. and S.C. .

RESULTS

Lithium regulation of adenylate cyclase

In initial experiments, lithium regulation of adenylate cyclase expression was studied by Northern blotting. Rats were treated chronically with lithium under therapeutically relevant conditions -- for 4 weeks with final serum levels of approximately 1 mM. Such lithium treatment increased mRNA levels for both type 1 and type 2 adenylate cyclase by 50-60% in cerebral cortex (fig. 4A).

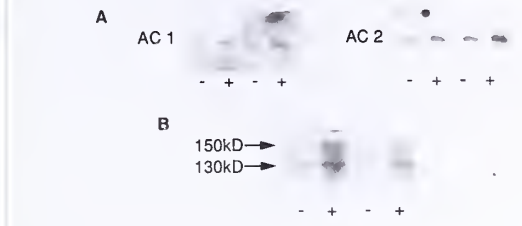

Figure 4. Autoradiograms illustrating chronic lithium regulation of adenylate cyclase expression in rat cerebral cortex. Rats were treated chronically with lithium, under therapeutic conditions, as described in Methods. Total RNA or membrane fractions, isolated from cerebral cortex of control (-) and drug-treated (+) animals, were then subjected to Northern blotting (A) or immunoblotting (B) (see Methods). The results demonstrate that chronic lithium increases levels of adenylate cyclase mRNA and protein. Levels of mRNA for type 1 (AC1) and type 2 (AC2) adenylate cyclase were, respectively: 159 $\pm$ 4%* and 150 $\pm$ 6%* of control $\pm$ s.e.m. (N=6). Levels of adenylate cyclase immunoreactivity were difficult to quantify due to the low levels of the enzyme present in control cortex; however, the effect shown in the figure was consistent among 6 control and 6 treated animals. *$P < 0.05$ by χ^2 test.

In contrast, treatment of rats with lithium for 6 days (with final lithium levels of approximately 1 mM) or for 4 weeks but at a lower dose (with final lithium levels of approximately 0.5 mM) failed to alter mRNA levels of either form of adenylate

cyclase, although there was a tendency for the short-term treatment to increase expression of the type 1 enzyme (Fig. 5).

To determine whether lithium regulation of adenylate cyclase mRNA was associated with equivalent regulation of enzyme protein, levels of adenylate cyclase immunoreactivity were studied by immunoblotting. Chronic treatment of rats with lithium under therapeutic conditions produced a dramatic induction of adenylate cyclase immunoreactivity (Fig 4B). The 150 kDa band has been identified in previous studies as a calmodulin-insensitive form of adenylate cyclase and most likely represents the type 2 enzyme. The 130 kDa band could represent the type 1 form of the enzyme, which has been reported to migrate between 115 and 135 kDa (39,41,42).

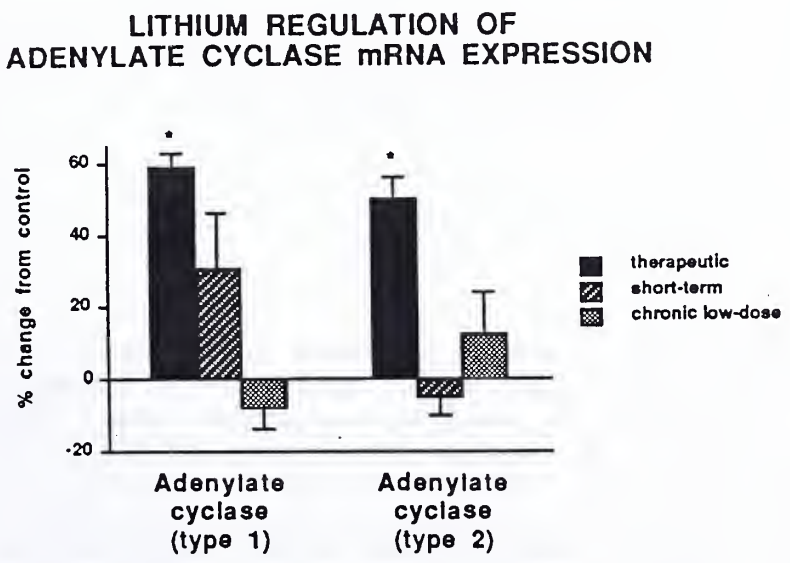

Figure 5. Time- and dose-dependence of lithium regulation of adenylate cyclase mRNA. Rats were treated with lithium under three conditions: 1) therapeutic: four weeks with final lithium levels of ~1 mM; 2) short-term: six days with final lithium levels of ~1.0 mM; or 3) chronic low-dose: four weeks at a lower dose with final lithium levels of ~0.5 mM (see Methods). Total RNA, isolated from cerebral cortex of control and drug-treated animals, was then subjected to Northern blotting for type 1 and type 2 adenylate cyclase as described in Methods. Data are expressed as mean $\pm$ s.e.m. and represent the results obtained from 6-12 control and lithium-treated rats. *$P < 0.05$ by χ^2 test.

Lithium regulation of G-proteins

Next we studied lithium regulation of G-protein expression in cerebral cortex. Administration of lithium under therapeutic conditions (i.e., 4 weeks at approximately 1 mM) decreased mRNA levels of $G_{i\alpha 1}$ and $G_{i\alpha 2}$ by approximately 20% (Fig. 6A). In contrast, such lithium treatment did not affect mRNA levels of the other G-protein subunits studied, which included $G_{s\alpha}$, $G_{o\alpha}$, and G_{β} (Fig. 6A).

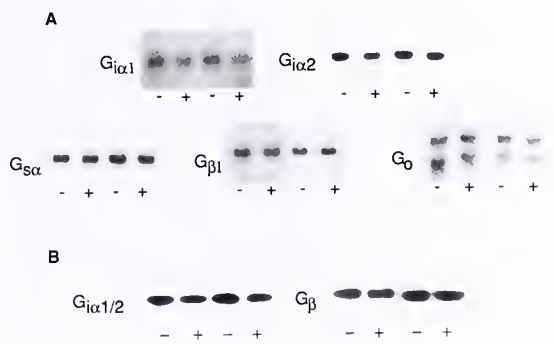

Figure 6. Autoradiograms illustrating chronic lithium regulation of G-protein expression in rat cerebral cortex. Rats were treated chronically with lithium, under therapeutic conditions, as described in Methods. Total RNA or crude homogenates, isolated from cerebral cortex of control (-) and drug-treated (+) animals, were then subjected to Northern blotting (A) or immunoblotting (B) (see Methods). The results demonstrate that chronic lithium decreases levels of $G_{i\alpha 1/2}$ mRNA and protein, with no effect seen on other G-protein subunits. Levels of G-protein mRNA were normalized to levels of cyclophilin mRNA (Cyc), which were not altered by chronic lithium. Levels of G-protein mRNA were: $G_{i\alpha 1}$, 76 $\pm$ 5% (12)*; $G_{i\alpha 2}$, 82 $\pm$ 2% (5)*; $G_{o\alpha}$, 108 $\pm$ 9% (6); $G_{s\alpha}$, 103 $\pm$ 5% (6); G_{β}, 103 $\pm$ 9% (6) of control $\pm$ s.e.m. (N). Levels of G-protein immunoreactivity were: $G_{i\alpha 1/2}$, 83 $\pm$ 3% (10)*; G_{β}, 102 $\pm$ 7% (5) of control. Lithium regulation of G-protein mRNA and immunoreactivity illustrated in the figure was replicated in two separate experiments.
*$P < 0.05$ by χ^2 test.

The effect of lithium on $G_{i\alpha 3}$ could not be assessed due to the very low resting levels of this G-protein subunit in cerebral cortex. Lithium down-regulation of $G_{i\alpha}$ mRNA was only found under therapeutic conditions; no effects were seen in response to short-term or chronic low-dose treatment (Fig. 7).

To investigate whether chronic lithium-induced decreases in $G_{i\alpha 1/2}$ mRNA were associated with equivalent changes at the protein level, lithium regulation of $G_{i\alpha}$ immunoreactivity was investigated. It was found that chronic (therapeutic) lithium administration decreased levels of $G_{i\alpha 1/2}$ immunoreactivity by nearly 20% but had no effect on levels of G_{β} immunoreactivity in cerebral cortex (Fig. 6B).

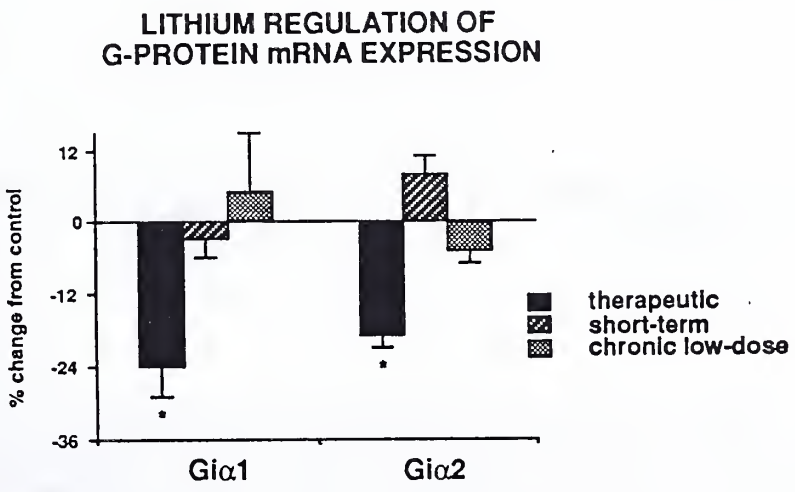

Figure 7. Time- and dose-dependence of lithium regulation of $G_{i\alpha}$ mRNA. Rats were treated with lithium under three conditions, therapeutic, short-term, or chronic low-dose, as described in the legend to Figure 5. Total RNA, isolated from cerebral cortex of control and drug-treated animals, was then subjected to Northern blotting for $G_{i\alpha 1}$ and $G_{i\alpha 2}$ (see Methods). Data are expressed as mean $\pm$ s.e.m. and represent the results obtained from 6-12 control and lithium-treated rats. *$P < 0.05$ by χ^2 test.

Lithium does not regulate PKA or PKC

In order to study possible lithium regulation of other signal transducing proteins we conducted Northern blotting studies for the PKA catalytic subunit Cα and the regulatory subunit RIβ. It was found that chronic lithium did not significantly affect cortical mRNA levels of either of these PKA subunits (Fig. 8A). Similar studies were conducted on PKC subtypes β1 and γ. There was no evidence of lithium regulation of these relatively abundant PKC isozymes at the mRNA level (Fig. 8B).

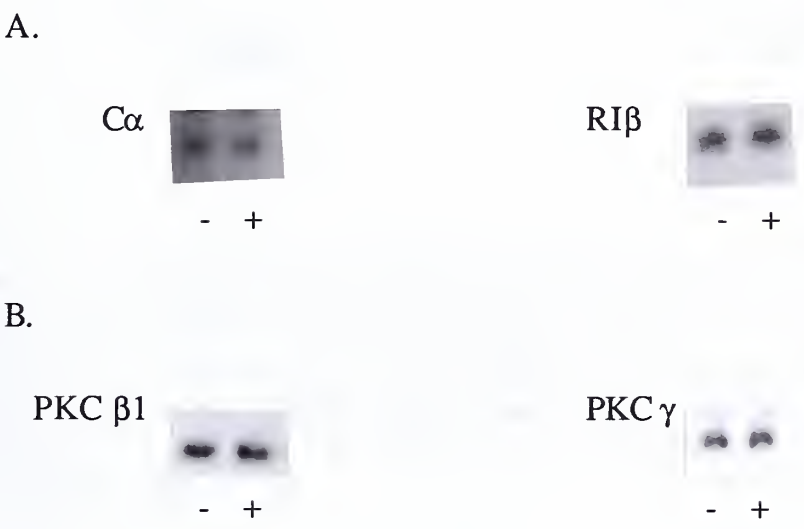

Figure 8. Autoradiograms illustrating that chronic lithium does not regulate PKA (A) or PKC (B) expression in rat cerebral cortex. Rats were treated chronically with lithium, under therapeutic conditions, as described in Methods. Total RNA isolated from cerebral cortex of control (-) and drug-treated (+) animals were then subjected to Northern blotting (see Methods). A. The results demonstrate that chronic lithium does not significantly affect PKA subunits Cα and RIβ mRNA levels in cortex (94 ± 4% (6) and 105 ± 3% (6) respectively). B. Chronic lithium did not significantly alter PKC subtypes β1 and γ mRNA levels in cortex (107 ± 6% (6) and 95 ± 7% (6) respectively).

Lithium does not regulate nuclear proteins c-fos or sp1

Our final studies investigated a potential mechanism for lithium regulation of adenylate cyclase and G-protein gene expression -- specifically, possible chronic lithium regulation of the transcriptionally active nuclear proteins c-fos and sp1. Chronic lithium did not significantly alter the cortical mRNA levels of either of these transcriptional regulatory proteins (Fig. 9).

Figure 9. Autoradiograms illustrating that chronic lithium does not regulate c-fos or sp1 expression in rat cerebral cortex. Rats were treated chronically with lithium, under therapeutic conditions, as described in Methods. Total RNA isolated from cerebral cortex of control (-) and drug-treated (+) animals were then subjected to Northern blotting (see Methods). The results demonstrate that chronic lithium does not significantly affect c-fos or sp1 mRNA levels in cortex (96 $\pm$ 5% (6) and 107 $\pm$ 8% (6) respectively).

DISCUSSION

Internal controls on quantitative Northern blotting

As Northern blotting is a complex procedure with many opportunities for error including RNA degradation, incomplete transfer of RNA, and non-uniform interactions between the RNA and the ^{32}P-DNA probe, we first demonstrated that our techniques were quantitatively accurate. Successive lanes were loaded with 10 ug, 9 ug, 8 ug etc. of RNA and the gel underwent electrophoresis and Northern blotting as described in Methods. Quantitative analysis revealed a linear decrease in radioactive signal (as measured by betascope) across lanes corresponding to the decrease in total RNA. These experiments indicated that we could reliably compare adjacent lanes to quantify drug induced changes in the radioactive signal for the probed mRNA.

Lithium regulation of adenylate cyclase and $G_{i\alpha}$

The results demonstrate that chronic lithium treatment increases levels of adenylate cyclase mRNA and protein in rat cerebral cortex. Both the calmodulin-sensitive (type 1) and calmodulin-insensitive (type 2) forms of adenylate cyclase were regulated by chronic lithium administration. These findings demonstrate that adenylate cyclase expression, like that of other signal transduction proteins, can be regulated in the nervous system. Moreover, the results indicate that lithium regulation of adenylate cyclase expression may contribute to its clinical efficacy, which also requires chronic exposure to therapeutic concentrations.

Previous reports have focused on inhibition of adenylate cyclase by acute and chronic lithium. Acute lithium is thought to inhibit adenylate cyclase activity at least in part through a direct action on the catalytic unit of the enzyme. As previously discussed, lithium may compete for Mg^{++} binding sites on the enzyme, which are required for its catalytic activity (15). Studies on chronic lithium have reported decreased levels of

neurotransmitter-, guanine nucleotide-, and forskolin-stimulated adenylate cyclase activity in membranes or slices of cerebral cortex (8,9). These inhibitory effects appear to represent persistence of the acute inhibitory actions of lithium on adenylate cyclase. However, it was also noted in these studies that chronic lithium increased basal levels of cAMP production by approximately 2-fold in both cerebral cortical membranes and slices of cerebral cortex (8,9), and we have corroborated these observations by demonstrating that, under our treatment conditions, chronic lithium increases basal levels of adenylate cyclase activity in cerebral cortical membranes (unpublished observations). These observations are consistent with the increased levels of adenylate cyclase mRNA and protein reported here and indicate that increased basal levels of cAMP production and of adenylate cyclase catalytic activity could be due to an increase in enzyme expression. In this context, up-regulation of adenylate cyclase expression by chronic lithium can be viewed as a compensatory, homeostatic response to persistent acute inhibition of the enzyme. The increases observed in adenylate cyclase expression and basal activity appear to predominate over the persistent acute inhibition of the enzyme in the chronic lithium-treated state: Masana et. al. recently found that chronic lithium produces a >2-fold increase in basal extracellular levels of cAMP in cerebral cortex as measured by *in vivo* microdialysis (43). These microdialysis studies demonstrate the functional physiologic correlate of the lithium induced increase in adenylate cyclase expression that we have described.

Previously, lithium has also been shown to interfere with G-protein function acutely (16). Lithium inhibition of GTP binding may be associated with an attenuation in the ability of neurotransmitters to regulate adenylate cyclase activity. In the present study, we have demonstrated that chronic lithium decreases $G_{i\alpha}$ expression in cerebral cortex, with no effect observed on other G-protein subunits. This decreased expression of $G_{i\alpha}$ represents an additional action of lithium on

G-proteins which may contribute to its therapeutic efficacy as this effect was observed in response to chronic lithium only. The observed decrease in levels of $G_{i\alpha}$, without a change in $G_{s\alpha}$ or G_{β}, would be expected to decrease the ability of inhibitory neurotransmitters to regulate adenylate cyclase activity. This action, along with increased adenylate cyclase expression, would produce a concerted, and possibly synergistic, up-regulation of the adenylate cyclase system in the chronic lithium-treated state. In sum, chronic lithium treatment appears to have two separate, but related effects -- (i) the persistence of an acute inhibition of agonist stimulated cAMP production, and (ii) a compensatory increase in basal (ie.unstimulated) adenylate cyclase activity. We propose that the molecular basis of this second effect is the chronic lithium induced change in adenylate cyclase and $G_{i\alpha}$ expression described herein.

Although the effect of chronic lithium on $G_{i\alpha}$ expression was relatively small (about 20%), a reduction of this magnitude would be expected to exert significant functional consequences. It has been shown that inhibition of $G_{i\alpha}/G_{o\alpha}$ by 10-15% (by pertussis toxin administration) decreases by 40-50% the ability of neurotransmitters to produce their electrophysiological effects on specific neuronal cell types, whereas inhibition of the G-proteins by 40-50% leads to an almost complete blockade of the electrophysiological responses (45).

Recent studies on chronic lithium regulation of G-proteins have confirmed our findings -- specifically, that chronic lithium decreases $G_{i\alpha 1}$ and $G_{i\alpha 2}$ mRNA in cortex (44). However, in contrast to our studies, this report cited a 25% decrease in $G_{s\alpha}$ following chronic lithium (44). It is important to note that this study did not provide any data on corresponding changes in protein levels, nor did it provide evidence of any G-proteins which are not regulated by lithium. It is possible that the discrepancy in $G_{s\alpha}$ findings can be attributed to the use of different lithium protocols or different species of rat -- our

studies were conducted on Sprague-Dawley rats whereas the other studies were conducted on Wistar rats. Finally, it is also possible that the discrepancy in $G_{s\alpha}$ findings is due to experimental variation or error.

Our discussion has focused on lithium regulation of "upstream" components -- G-proteins and adenylate cyclase -- in the cAMP cascade. However, it is equally important to consider the final common effector in this system, PKA. Recent cloning experiments have discovered two distinct catalytic subunits, Cα (expressed in periphery and brain) and Cβ (preferentially expressed in brain), and 4 distinct regulatory subunits RIα, RIβ, RIIα, and RIIβ (46). Recent studies on lithium regulation of PKA activity in cortex reveal that there is no change in enzyme activity after chronic treatment (47). This would suggest that either there is no change in the level of catalytic subunit, or that any change in the catalytic subunit is compensated by similar changes in the regulatory subunit. In an elegant set of experiments to study co-regulation of catalytic and regulatory PKA subunits, McKnight et. al. transfected 3T3 fibroblasts with a Cα expression vector containing an inducible metallothionein promoter. Upon Zn^{++} administration, Cα mRNA and protein rapidly rose to greater than five times basal levels (46). Interestingly, there was a significant compensatory increase in RI protein with no change in RII (46). It appears that there is a pre-programmed mechanism to sequester increased Cα expression with increased RI expression (46). This homeostasis at the level of gene expression may prevent cells from producing excess catalytic subunit, which would be constitutively active in the relative absence of regulatory subunits (46). It is unclear whether these factors have relevance to *in vivo* cortex conditions.

In order to study possible chronic lithium regulation of PKA we performed Northern blotting studies using cDNA probes for several PKA subunits. Our preliminary data suggest that neither Cα nor RIβ mRNA levels are regulated by chronic lithium. Studies on the other subunits were difficult due to

either low mRNA levels or high levels of non-specific ^{32}P-DNA binding (ie. background). In the absence of positive data at the mRNA level we were dissuaded from attempting immunoblotting studies on chronic lithium regulation of PKA.

Possible mechanisms of lithium regulation of gene transcription

Our findings of chronic lithium regulation of adenylate cyclase and $G_{i\alpha}$ expression raise the question of a possible molecular mechanism for lithium control of gene transcription. Specifically, how might lithium interact with the 5'-transcriptional control region of these genes. In consideration of lithium's well documented effects on inhibiting cAMP production and PIP_2 hydrolysis it seems highly plausible that lithium affects PKA or PKC regulated gene transcription. Under this hypothesis, lithium would decrease cAMP levels, and thereby decrease PKA phosphorylation of CREB. This change in CREB phosphorylation might directly regulate transcription of genes with cAMP regulatory elements. We have conducted several computer based searches for cAMP regulatory elements in the 5'-non-coding regions of adenylate cyclase type 1 (the sequence for type 2 remains unpublished), $G_{i\alpha 1}$ and $G_{i\alpha 2}$ which have confirmed the absence of such sequences (17,31). However these searches were confined to small regions upstream of the start site as these published gene sequences did not extend very far into the 5'-non-coding region (17,31).

Another interesting possibility is that lithium, by inhibiting second messenger formation, regulates the expression of a secondary transcriptionally active protein and that this protein regulates adenylate cyclase, $G_{i\alpha 1}$ and $G_{i\alpha 2}$ transcription. Interestingly, all three genes contain multiple "GC" boxes in the 5'-non-coding region -- the recognition element for the transcriptional regulatory protein sp1 (17,31,48). Accordingly we searched the 5'-non-coding region of sp1 for TRE and CRE sites. Surprisingly we found an estrogen hormone regulatory element whose dyad half-site is almost

identical to the TRE and CRE: The ERE half-site (underlined) is followed by 5'-CA-3' to form 5'-TGACCTCA-3' . This sequence is strikingly similar to both the CRE (5'-TGACGTCA-3') and the TRE (5'-TGACTCA-3'). Since both CREB and AP1 exhibit promiscuity in DNA binding (ie. both proteins are known to bind sequences similar to their recognition elements), it is plausible that sp1 is regulated by CREB or AP1 action at this site. In order to test our hypothesis that lithium controls sp1 expression (possibly via alterations in CREB or AP1 activity), we performed Northern blotting studies on chronic lithium treated rat cortex. Unfortunately, sp1 mRNA is exceedingly rare (like many other transcriptionally active nuclear proteins) and it was therefore difficult to measure its presence by the forestated techniques despite: (i) using excess amounts of total RNA (up to 75 ug per lane) , (ii) doubling the specific activity of the radiolabelled probe by random labelling with ^{32}P-dATP and ^{32}P-dGTP, and (iii) exposing the autoradiogram for up to 14 days. With these modifications we produced preliminary results which suggest that sp1 is not regulated by lithium. Despite these technical difficulties we continued to study estrogen regulation of sp1. We believe these studies may provide the first demonstration of a steroid hormone directly regulating a major transcriptional protein. In sum, the mechanism by which lithium regulates gene expression of specific genes has been elusive and remains to be defined.

As we have discussed to this point, lithium regulation of adenylate cyclase and $G_{i\alpha}$ mRNA and protein is consistent with regulation at the level of gene expression, although alternative mechanisms, such as regulation of mRNA stability or translation, cannot be ruled out. As adenylate cyclase and G-proteins are regulated by lithium acutely, it will be important to determine whether other acute targets of lithium (for example, components of the phosphatidylinositol system) are also regulated by chronic lithium treatment. Our results suggest that PKC subtypes β_1, δ (unpublished observation), and γ are not regulated by chronic lithium. These findings are

supported by recent studies which have revealed that chronic lithium does not affect PKC activity in cortex (47). In addition, studies conducted elsewhere have determined that lithium does not affect the levels of IP_3-receptor (49). Future studies should address the intriguing possibility of lithium regulation of PLC. Such studies are particularly interesting in light of the finding that chronic lithium followed by 18 hours of withdrawal induces an increase in muscarinic stimulated PIP_2 hydrolysis (21). One possibility is that in response to lithium's acute inhibition of PIP_2 hydrolysis, there is a compensatory increase in PLC or the G-protein which couples the receptor to PLC.

The results of the present study indicate that some of the chronic effects of lithium on brain function may be mediated by alterations in adenylate cyclase and G-protein expression. This view is underscored by the fact that these changes were fully dependent on a therapeutic dose and time-course. Neither short term lithium or chronic low dose lithium induced these changes in adenylate cyclase and $G_{i\alpha}$. Through the study of chronic lithium regulation of signal-transducing proteins a more complete understanding will emerge concerning the mechanisms by which lithium produces its clinical antimanic and antidepressant actions. This understanding should help to elucidate the molecular basis of manic-depressive disorder.

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